Recent Advances in the Applications of Radioisotopes in Drug Metabolism, Toxicology and Pharmacokinetics

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Abstract: Radioisotopes have proven to be an indispensable tool in biomedical research and have played a pivotal role in the investigation of absorption, distribution, metabolism and excretion (ADME) properties of new chemical entities over the past several decades. The main advantage of using radioisotopes in studying the disposition of new drug candidates is the ease of detection and the achievement of high sensitivity, especially when compounds with high specific activity are used. The recent advances and applications of radioisotopes in designing and conducting ADME studies and its impact in the field of drug metabolism and pharmacokinetics are discussed in this review.

INTRODUCTION

Background and Objective

The use of radioisotopically labelled drug candidates remains an indispensable tool in pharmacological research. Since the introduction of the first radioactive isotope (^{14}C) in the late 1940's, the use of radioactive isotopes has undergone a dramatic expansion over the years. This is partly due to the development of new technology for analyzing radiolabelled compounds as well as the growing demand for their use as tracers to conduct studies in humans. The diversity of applications of radioactive isotopes in pharmacological and biomedical research may be illustrated by reference to several review articles that have been published [1-9].

Radioisotopes have been used in all areas of biomedical research because of the ease with which the radioactivity can be detected and quantified utilizing relatively inexpensive and easy liquid scintillation counting techniques. A more compelling reason for their use in the drug metabolism area is the great sensitivity that can be achieved, especially when compounds with high specific activity are used. The high degree of accountability obtainable in studies with radiolabelled compounds and/or their metabolites has made it possible to track these compounds in biological systems. The primary objective of this review is to summarize the progress made in the use of radioisotopes in designing and conducting the absorption, distribution metabolism and excretion (ADME) studies. The literature in this field is extensive and hence an attempt has been made to focus on the role and the impact of radioactive isotopes in the field of drug metabolism, toxicology and pharmacokinetics.

Labelling of a new drug candidate with a radioactive tracer helps in studying the ADME characteristics of these candidates and is often essential for the success and timely completion of such studies [10]. A schematic diagram of ADME processes following an oral (po) and an intravenous (iv) dose of a radiolabelled drug are illustrated in Fig. **1**. The use of non-tracer analytical techniques such as gas chromatography (GC) or high-performance liquid chromatography (HPLC) with UV or fluorescence detection are valuable for *in vitro* and *in vivo* drug metabolism studies. Recent technological advances in drug quantification, using mass spectrometry have greatly facilitated this analysis. However these techniques seldom provide the volume of information that can be obtained using radioisotopes. New guidelines recently proposed by regulatory agencies require studies such as tissue distribution, and plasma and tissue protein binding which can only be obtained using radiolabelled compounds [11]. Dain and coworkers have published a regulatory and industrial perspective on the use of carbon-14 and tritium tracers in human ADME studies [12]. Also, a comprehensive review summarizing clinical drug

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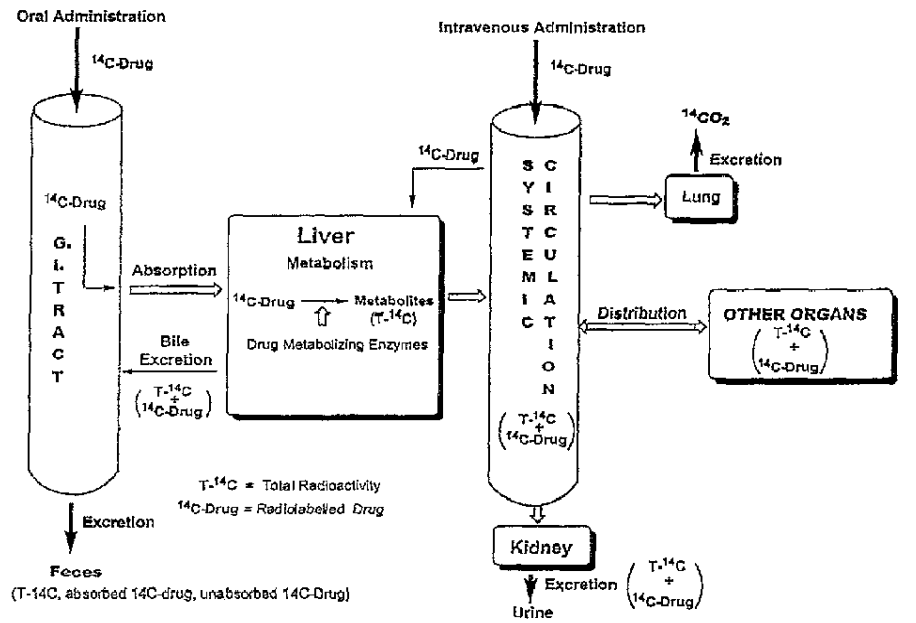


Fig. (1). A schematic diagram of the ADME processes following an oral (po) and intravenous (iv) dose of a radiolabelled drug.

ADME = Absorption, Distribution, Excretion and Metabolism.

metabolism studies has been published recently [13, 14]. These reviews also describe the guidelines and objectives of conducting ADME studies using radiolabelled compounds in humans.

Some important parameters to be considered in designing ADME studies are: choice of the radioisotope, position of the radiolabel in the molecule, the specific activity, radiochemical purity and the complexity of tracer synthesis. The criteria and the strategies for the synthesis of radiolabelled compounds and other synthetic aspects of radiolabel compounds have been covered elsewhere in this volume and will not be discussed here. The position of radiolabel merits careful consideration and the choice is governed by the proposed use of the radiolabelled compound. For metabolism studies, the label is generally introduced into a metabolically stable position such as an aromatic or alicyclic ring system. Computer programs, such as computer assisted metabolism prediction (CAMP) with a large data base of metabolic transforms, can be useful in the prediction of biologically stable positions of the compound, thus facilitating selection of the most suitable precursor for introducing of the label [15].

Choice of Radioisotopes

Carbon-14 is the isotope of choice and the most extensively employed in ADME studies. In some cases, where high specific activity is required tritium is often preferred and is placed in a non-exchangable position. Several other isotopes such as, ^{35}S, ^{32}P, ^{125}I, ^{131}I, and ^{59}Fe have found applications in studying the metabolic disposition of compounds. Table 1 shows the most common radioisotopes used in drug metabolism studies. Both, ^{35}S and ^{32}P have been invaluable in studying the pharmacokinetics and metabolism of antisense oligonucliotide phosphorothiorates [16, 17]. The pharmacokinetics and metabolism of GEM91®, a oligodeoxynucleotide phosphothiorate, was investigated in cynomolgus monkey following intravenous infusion using [^{35}S] labelled phosphothiorate [18]. ^{59}Fe has found limited application in the labelling of erythrocytes and has been used for assessing the blood loss after administration of nonsteroidal antiinflammatory agents [19]. It also has been employed in studying the absorption and the biological fate of physiologically important elements. ^{125}I and ^{131}I are often preferred for bioassays which are based on immunological or receptor binding principles [20,

21]. Also, [^{125}I] labelled CC49 monoclonal antibody has been used to study its pharmacokinetics in subjects with colon cancer [22].

Table 1. Radioisotopes Used in ADME Studies and their Half-lives [7]

Radioisotopes	Half-lives
^{14}C	5760 years
^{3}H	12 years
^{35}S	87 days
^{32}P	14 days
^{33}P	25 days
^{125}I	60 days
^{131}I	8 days
^{59}Fe	45 days

USE OF RADIOISOTOPES IN *IN VIVO* STUDIES

Absorption

Absorption is defined as a process by which a compound and its metabolites are transferred from the site of application to systemic circulation and reflects the total exposure of drug-related material to animals and humans as shown in Fig. 1. The knowledge of the rate and extent of absorption is essential for a proper safety evaluation and development of new entities. Radiotracers have been advantageous in studying absorption and have been employed for this purpose for several years.

In the absence of a radiolabelled compound, the bioavailability of a new candidate, which represents the fraction of the administered drug reaching systemic circulation, is assessed by quantifying the parent drug using non-tracer analytical techniques. The main drawback however, is that the systemic bioavailability does not truly assess the absorption of the drug, since several factors (at the site of application) can influence its exposure. The equations to differentiate absorption from bioavailability are shown in Fig. 2. For instance, following an oral administration the drug has to pass through the hepato-portal system and is subjected to metabolism before entering the general circulation (first pass effect). This may result in differences between the actual amount of drug-related material absorbed and the fraction of the circulating unchanged drug. Determination of the amount of drug-related material absorbed proves to be quite cumbersome using unlabelled compound and depends on the knowledge and the availability of authentic standards of the metabolites and development of an assay for the metabolites. The use of radiolabelled drug on the other hand, allows an accurate quantitation of drug and the drug-related material that is absorbed without standards and elaborate assay development.

$$\text{Absorption} = \frac{\text{Exposure of } \begin{matrix}\text{T-}^{14}\text{C} \\ + \\ ^{14}\text{C-Drug}\end{matrix} \text{ after po dose}}{\text{Exposure of } \begin{matrix}\text{T-}^{14}\text{C} \\ + \\ ^{14}\text{C-Drug}\end{matrix} \text{ after iv dose}}$$

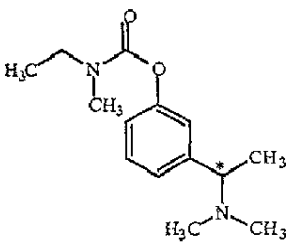
or

$$\left\{ \begin{matrix}\text{T-}^{14}\text{C} \\ + \\ ^{14}\text{C-Drug}\end{matrix} \text{ in bile} \quad \begin{matrix}\text{T-}^{14}\text{C} \\ + \\ ^{14}\text{C-Drug}\end{matrix} \text{ in urine} \right\} \text{after po dose}$$

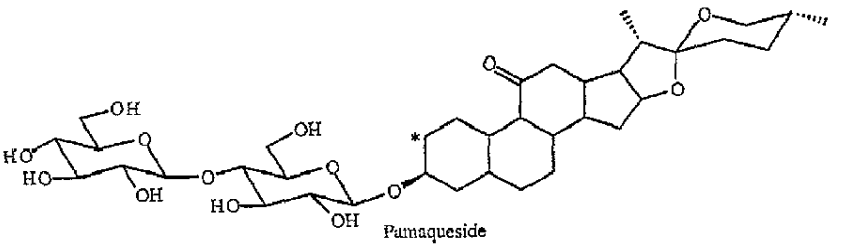
$$\text{Bioavailability (F)} = \frac{\text{Exposure of } ^{14}\text{C-Drug after po dose}}{\text{Exposure of } ^{14}\text{C-Drug after iv dose}}$$

Fig. (2). Determination of absorption and bioavailability after po and iv dose of a radiolabelled drug [also refer to Fig. 1].

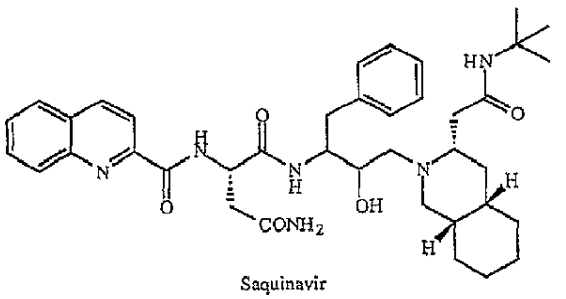
Tse and Laplanche have demonstrated the differences in the amount of drug-related material absorbed and the systemic bioavailability of the parent drug with [^{14}C]SDZ ENA 713, an acetylcholinesterase inhibitor [23] (Fig. 3 shows the chemical structures of most of the compounds discussed in this report. Wherever possible, the site of the radiolabel in the molecule is shown by an asterisk). The study compared the pharmacokinetics of SDZ ENA 713 in mini-pigs following intravenous, oral and dermal administration. The groups of mini-pigs received a single intravenous (iv) dose of 0.1 mg/kg, single po dose of 1.0 mg/kg or topical doses of 18 mg or 54 mg of [^{14}C] SDZ ENA 713. After each dose, blood, urine and feces were collected at designated intervals for 7 days. The amount of radioactivity in the biological samples was determined using conventional liquid scintillation counting techniques and the parent drug and a metabolite were measured by an HPLC assay. Comparison of the exposure of radioactivity following iv and po doses indicated that [^{14}C]SDZ ENA 713 was rapidly (T_{max} = 0.83 h) and efficiently absorbed (93%). However, analysis of



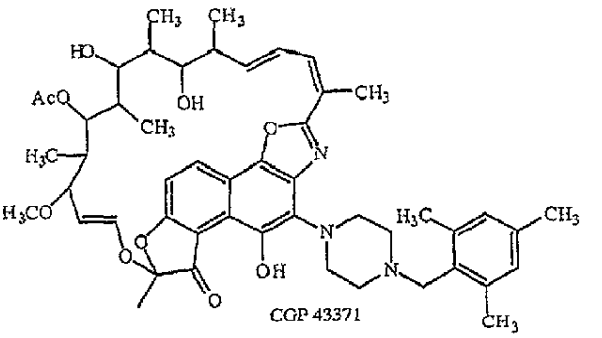
SDZ ENA 713



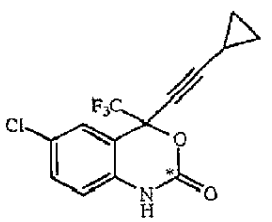
Pamaqueside



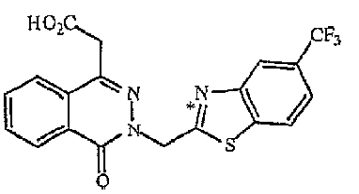
Saquinavir



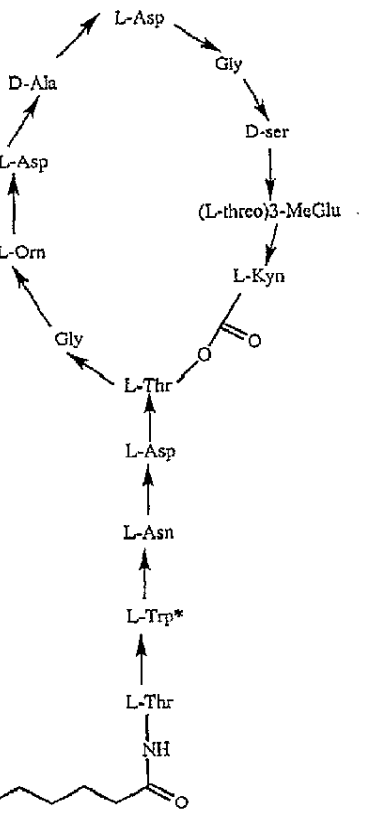
CGP 43371



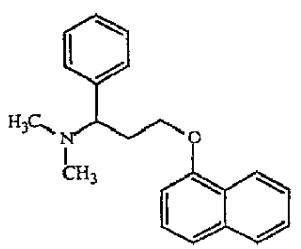
Efaviren 2



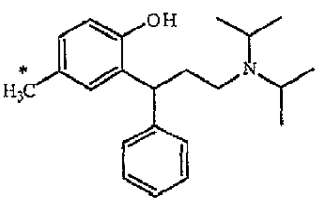
Zopolrestat



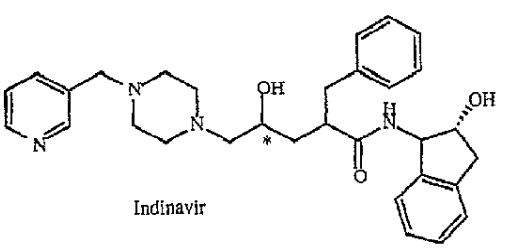
Daptomycin



Dapoxetine



Tolterdine



Indinavir

PM3006723328

(Fig. 3). contd.....

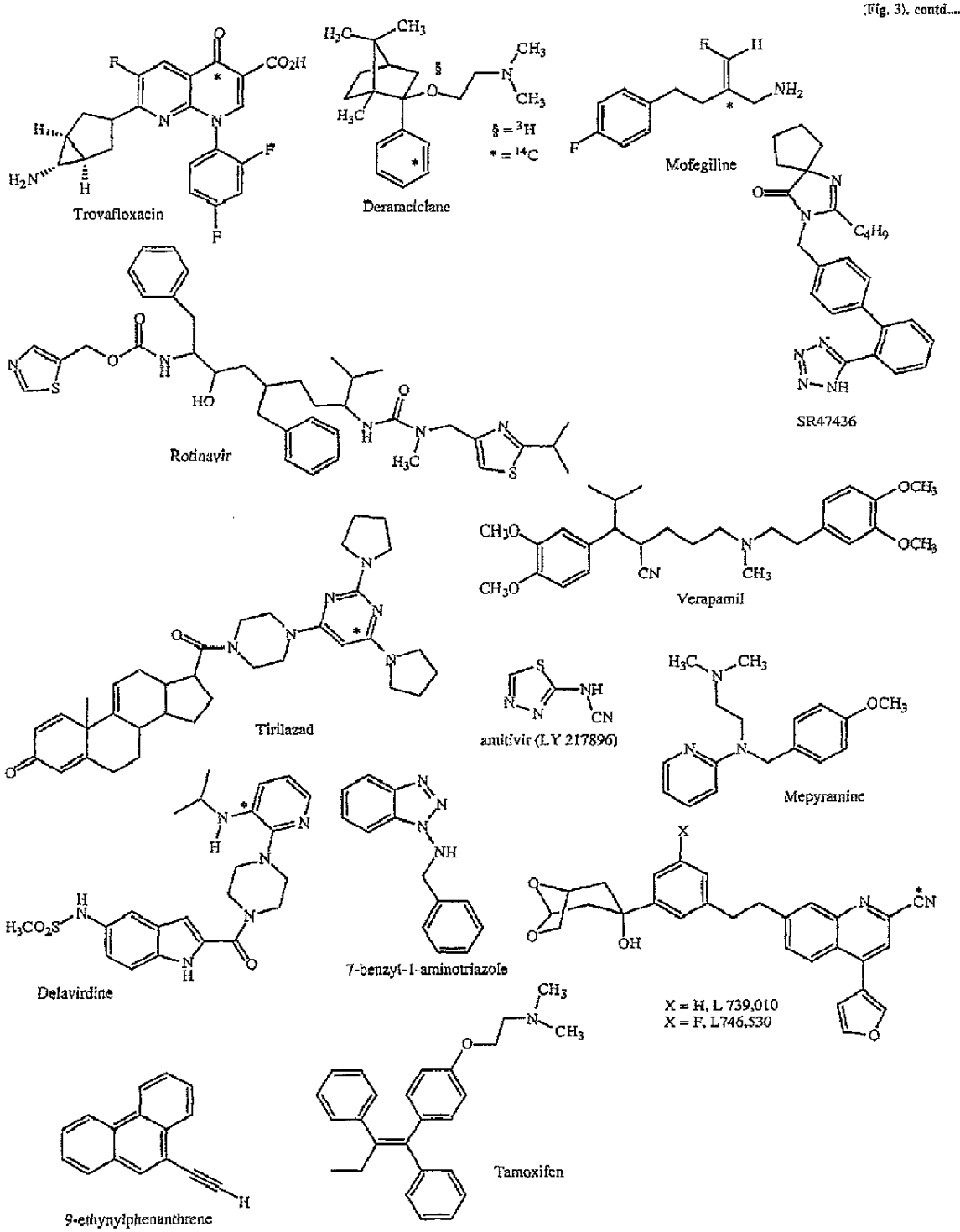


Fig. (3). Chemical structures of compounds (asterisk indicates the position of the radiolabel).

plasma levels of the parent drug following these two routes suggested that the bioavailability of the parent drug was low (0.5%), apparently due to extensive first pass effect. After dermal administration of [^{14}C]SDZ ENA 713, the absorption was only 8%. Since most of the drug administered by this route reached systemic circulation intact, the bioavailability of SDZ ENA 713 was 20 times greater than that following an oral dose.

In our laboratory, the use of radiolabel has helped us unveil the effect of surgery on the absorption of pamaqueside [24]. In an attempt to understand the absorption and metabolism of pamaqueside, non-cannulated and bile-duct cannulated Long-Evans rats were orally administered [^{14}C]pamaqueside at 100 mg/kg. The urine and feces were collected from the non-cannulated rats while bile, urine and feces were collected from the bile-duct cannulated rats. The collected biological samples were analyzed by conventional liquid scintillation counting to determine the amount of radioactivity present in the biological samples. Only 0.1% of the radioactivity was excreted in the urine of the non-cannulated rats as shown in Table 2. Interestingly, about 17% of dose was recovered in the bile and urine of the bile-duct cannulated rats. On the other hand, analysis of the biological matrices for drug concentrations in the surgerized as well as non-surgerized animals indicated poor oral bioavailability of unchanged pamaqueside. Thus, the presence of radiolabelled pamaqueside was useful in demonstrating the effect of surgery on absorption of pamaqueside in bile-duct cannulated animals, even though the bioavailability of the drug was similar in both the groups of animals.

Table 2. Excretion of Radioactivity in Bile-duct Cannulated and Non-cannulated Long-Evans rats Following Oral Administration of 100 mg/kg [^{14}C]Pamaqueside [24]

Rats	% of Dose Excreted			
	Urine	Feces	Bile	Total
Bile-Duct Cannulated	5.8	76.2	11.1	93.1
Non-Cannulated	0.1	97.5	--	97.6

The percentage of radioactivity recovered in the bile and urine of bile-duct cannulated rats suggested that 16.9% of the dose was absorbed from the GI tract. The bioavailability (F) of pamaqueside was less than 1% in both the non-cannulated and bile-duct cannulated rats.

Drug absorption from the gastrointestinal tract is not a simple process and is influenced by several factors. In addition to the physicochemical properties of the drug that affect the rate of absorption many physiological factors hinder or enhance drug absorption. Food can have marked effects on drug absorption by altering the rate of gastric emptying [25]. Paracetamol has been widely used as a non-radioactive tracer for studies on drug absorption and its absorption has been used to measure the rate of gastric emptying [26]. Radioactive compounds have also been employed profitably as markers in studying the processes that affect oral absorption of drugs.

Studies with [^{14}C]saquinavir have shown that approximately 30% of the drug is absorbed from the gut and only 4% of this dose reaches the systemic circulation in humans [27]. Administration of saquinavir in fed state has been shown to substantially improve bioavailability. A pharmacoscintigraphic study using a water insoluble and non-adsorbable marker ^{153}Samarium oxide, in combination with the traditional pharmacokinetic techniques has been conducted to help elucidate the food effects observed with saquinavir [27]. The subjects were administered three capsules containing 200 mg saquinavir and ^{153}Samarium oxide (1 MBq) in a fasted and fed state. The non-radioactive ^{152}Sm was converted into a gamma emitting radioisotope [^{153}Sm] by irradiation in a neutron source following blending with the drug granulate and encapsulation. The scintigraphic images were recorded at approximately 10 min intervals until 12 hr post-dose using a gamma camera. Blood samples were also collected at different sampling times during the study in order to measure the levels of saquinavir. The study showed that rapid gastric emptying of intact capsules from the stomach in the fasted state, monitored scintigraphically, affected the absorption of saquinavir while bioavailability of saquinavir was significantly improved in the presence of food, due to prolongation of gastric emptying.

In another example, ^{153}Samarium oxide was used help elucidate possible causes for the variability in absorption of a hypolipidemic agent CGP43371 in healthy subjects [28]. The objective of the study was to determine the gastrointestinal locus of drug absorption by correlating the location of the radioactive marker with onset of drug absorption and to evaluate the gastrointestinal residence of CGP43371. In this study the subjects received a capsule of the drug and a capsule of ^{153}samarium oxide (100-300 μCi) as a non-absorbable marker of gastrointestinal transit and

fecal recovery of the drug. The gastrointestinal transit of ^{153}Sm was monitored by gamma scintigraphy and the drug in the fecal content and plasma samples was determined by HPLC. The onset of drug absorption in this study occurred 4 hr post dose, when ^{153}Sm was in the distal to terminal portion of the ileum. A positive correlation between the area under the drug curve and area under transit curve of gastric emptying showed that longer gastric residence improved oral absorption of CGP43371.

Several radioactive markers, other than ^{153}samarium oxide, have used in the investigation of gastrointestinal transit time. Recently, the effect of efavirenz, a potent HIV-1 reverse transcriptase inhibitor, on gastrointestinal transit time has been investigated in rats and monkeys using a non-absorbable, stable macromolecular probe, [^{14}C] polyethylene glycol 4000 (PEG 4000) [29]. In this study, a group of animals were dosed with [^{14}C] PEG 4000, with and without a challenge of efavirenz, 1 hr prior to the radioactive dose. Various parts of the gastrointestinal tract were ligated (at every 20 cm) upto the cecum, followed by excision of the segments. The radioactivity in these parts was then determined in a more conventional manner by homogenizing, drying and combusting the segments and counting the $^{14}CO_2$. The results showed that [^{14}C] PEG passed through the stomach rapidly in control rats but was 10 fold greater in efavirenz dosed rats.

Distribution

Radiolabelled compounds are invaluable in tissue distribution studies. Drug regulatory agencies require that tissue distribution studies be performed with animals as a prerequisite for estimating tissue exposure to radioactivity before administration of radiolabelled xenobiotics to human subjects in clinical studies. The concentrations of drug-related material (total radioactivity) in major tissues and organs reveal the degree of transient exposure of specific organs to the drug.

Tissue Dissection and Liquid Scintillation Counting (TD/LSC)

The TD/LSC is routinely used to quantify the distribution of xenobiotics to target tissues of animal species. Typically, a dose of radiolabelled drug is administered to rats, designated tissues and organs are collected from these animals after sacrificing them at various sampling times, and the radioactivity levels in the tissues are measured. The harvested tissues are analyzed for radioactivity, either by combustion in a sample oxidizer to generate $^{14}CO_2$, or by dissolving in a solubilizing agent followed by liquid scintillation counting. The determination of radioactivity at various time points allows one to estimate the half-life of the drug-related material in a particular tissue. Schneider and co-workers have utilized this technique to determine the tissue distribution of zopolrestat, an aldose reductase inhibitor, in rats [30]. [^{14}C]Zopolrestat was orally administered to rats and tissue samples were collected at different sampling times. The liver, illeum and the large intestine were the three organs that demonstrated the greatest exposure, as assessed by the area under the curve ($AUC_{0-\infty}$). The half-life in most tissues was 8-10 hr and was similar to the half-life in the plasma (Table 3).

Table 3. Pharmacokinetic Parameters of Radioactivity from [^{14}C]Zopolrestat in Male Rats after Single Oral Dose of 57 mg/kg [30]

Tissue	AUC0-∞ (ng equiv•hr/ml)	C_{max} (ng equiv/ml)	$t_{1/2}$ (hr)
Lung	798	47.2	8.1
kidney	1581	69.1	12.8
large intestine	3132	110	10.6
ileum	3007	211	10.5
jejunum	1059	53.1	9.4
liver	3529	146	22.2
stomach	1217	90.7	8.3
plasma	1836	105	8.2
whole blood	1049	55.0	8.6

Whole Body Autoradiography (WBA)

A more recent technique for the study of xenobiotic distribution is WBA [31]. This method provides an alternative approach for examining tissue radioactivity, but unlike TD/LSC, utilizes intact animals. First described by Ullberg in the early 1950's, it is the most widely employed to determine biological fate of radiolabelled drugs and other chemicals [32, 33]. WBA has several advantages over conventional TD/LSC for evaluating the distribution and fate of drugs. It provides an accurate and comprehensive visual survey of the major organs and tissues. It also detects the potential sites of accumulation that may be missed by TD/LSC where all tissues are not

routinely sampled. Generally, rats are orally dosed with a radiolabelled compound and euthanized at different times. The whole animal is frozen and sectioned and each sagittal section is then subjected to autoradiography. The organ/tissue radioactivity data obtained in the form of a photograph on an X-ray film shows the localization of the radiolabelled compound at appropriate times post-dose. The isotope concentrations in various organs of the body section are then quantified by comparative dosimetry [34-37]. [^{14}C] Glucose is used as an internal standard and control for the correct assessment of radioactivity. However, there are some doubts regarding the ability of X-ray films to quantitatively estimate the concentrations of the compound-derived radioactivity [38]. This is because over and under exposures of X-ray film is common and the dynamic range is limited to less than 2 orders of magnitude.

Alternative technologies such as phosphor imaging technology, also known as auto-radioluminography [39] has now been developed for the quantitative analysis of tissue concentrations in freeze-dried sections. Autoradioimmunography appears to have the greatest potential, offering an acceptable resolution, a broad dynamic range and sensitivity approximately 25 times higher than film autoradiography. A saggital section of a rat and distribution of the radiolabelled xenobiotic following WBA is shown in Fig. **4**. Toshiyuki and Furudate have reported the use of a new "liquid-solid scintillator" for the enhancement of autoradiograms with tritium agents [40]. The new scintillator originally in a soluble form quickly transforms to a solid homogenous film following exposure to room air. It enables the plates to be developed faster when compared to liquid scintillator and gives consistently high counting performance thus shortening the duration of exposure.

Several authors have compared the tissue concentrations obtained from the TD/LSC with WBA using either autoradiography or autoradioimmunography. Chay and Pohland have studied the tissue distribution of [^{14}C]daptomycin using the WBA and TD/LSC [41]. In this study, the authors have compared the two techniques for estimating the tissue concentrations of radioactivity (μg-equivalent/gram of tissue) after a single iv 10 mg/kg dose of [^{14}C] daptomycin in rats. A quantitative comparison of WBA using phosphor-imaging technique with TD/LSC, using [^{14}C] labelled xenobiotics has also been made by Potochoiba and co-workers [42]. This study also describes the advantages of using the phosphor-imaging technology over the X-ray films. The results from both these studies indicate that the tissue concentrations obtained by WBA, normalized using an internal standard, are comparable to those obtained by TD/LSC. WBA study also provided results of quantitative distribution of radioactivity to localized sites in organs not feasible by the latter technique. However, multiple exposures and several days were required to obtain quantitative information on the disposition of radiolabelled daptomycin using autoradiography compared to auto-radioimmunography.

Bernstein and co-workers have utilized tissue dissection and WBA techniques in a complimentary manner to study the disposition of dapoxetine in Fischer 344 rats [43]. [^{14}C] Dapoxetine, a potent

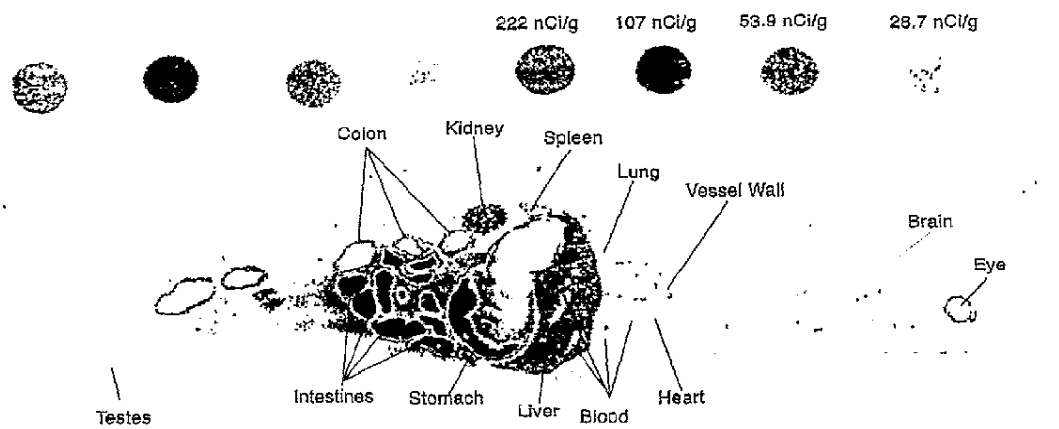


Fig. (4). A saggital section of a rat and tissue distribution of radiolabelled xenobiotic following whole body autoradiography (WBA) using phosphor imaging technology.

serotinin reuptake inhibitor, was orally administered to rats and radioactivity in the tissues was located using WBA. This helped to identify the organs not usually harvested in a tissue distribution study, namely the preputial and the harderian gland. Selected organs, based on WBA findings were dissected from rats and analyzed for radioactivity content by liquid scintillation counting and for parent drug and the metabolites by extraction and HPLC analysis.

Positron Emission Tomography (PET)

Recent advances in the noninvasive imaging techniques such as PET, single photon emission computed tomography (SPECT) and magnetic resonance imaging and spectroscopy (MRI and MRS) have greatly expanded the scope of pharmacokinetic measurements that can be performed in humans [44]. This technique has added a new dimension to the utility of short-lived radionuclides such as, ^{13}N, ^{11}C and ^{18}F in studying the disposition of drugs in humans. The quantitative nature of PET enables one to measure the tissue concentrations with high accuracy and the measurements are similar to the results obtained from direct quantification of radioactivity in tissue samples. Several examples illustrating the use of PET in the bio-distribution have been published recently [44]. The subject is covered in great details elsewhere in this volume and therefore will not be discussed here.

Studies of Plasma Protein Binding and Distribution into Red Blood Cells

Binding of drugs to plasma proteins has long been recognized to have a profound effect on the pharmacokinetic and pharmacodynamic properties of a drug. The ability of a drug to enter extravascular spaces is also determined by its distribution characteristics within the blood components such as erythrocytes. Because of the great sensitivity that can be achieved and the ease with which radioactivity can be measured, radioactive tracers of drugs are usually the method of choice for the determination of drug protein binding and distribution of the drug into the red blood cells. This is particularly useful for drugs that are extensively bound to proteins or erythrocytes when it may be difficult to measure the free (unbound) drug by non-radioisotope methods. Tritium (due to its high specific activity) is the label of choice when the free fraction of highly bound compounds is to be determined.

Blood-plasma distribution ratio and plasma protein binding are usually evaluated by *in vitro* studies. To determine the distribution of the drugs in erythrocytes, fresh blood is spiked with radioactive drug and incubated at 37 °C. Aliquots of blood, and plasma obtained from the remaining blood, are subjected to scintillation counting. The binding of the drug to plasma proteins can be determined by various techniques such as, equilibrium dialysis, ultrafiltration, ultracentrifugation and gel filtration. In all the above methods, the plasma is spiked with a radiolabelled drug and the aliquots of the sample are transferred to micropartition centrifuge tubes for the ultrafiltration and to the dialysis cell in the case of equilibrium dialysis. The samples are allowed to equilbrate and analyzed for radioactivity by liquid scintillation counting. As an example, *in vitro* serum protein binding of [^{14}C] tolterdine and its major metabolites was studied in humans and several animal species. The binding of the drug and its metabolites to human serum albumin and α-1-glycoprotein as well as red blood cells was also estimated in this study [45]. The objective was to investigate the apparent difference between the *in vivo* and *in vitro* potency of tolterdine and its metabolite and its relationship with the free fraction of the compounds.

Excretion

Excretion studies are generally conducted as a part of studying overall disposition of the drug. One of the primary objective of these studies is to show that the administered dose is readily excreted from the body, ideally after exerting its intended therapeutic effect. A mass balance study serves such a purpose. By quantitative collection of the excreta at sufficient frequency, it is not only possible to provide information of the overall excretion pattern, but also to allow an assessment of the rate of elimination. However, such a study is virtually impossible without the use of a radiolabelled compound.

The desired specific activity of the administered radioactive drug obviously depends on the dose to be used as well as the species studied. Doses of [^{14}C] of the order of 5 μCi/kg for the dog and 20 μCi/kg for the rat yield measurable radioactivity levels in the excreta and blood. Doses of [^{3}H] are usually two to three times higher than that of [^{14}C] owing to the lower counting efficiency. Typically, the animals are dosed intravenously or orally with a radiolabelled drug and placed in metabolism cages for collection of excreta. Urine and feces are collected over different sampling time e.g. 8, 24, 48, 72, 96 h, etc. Radioactivity is measured by

counting 100 to 500 µl of urine sample in vial containing scintillation cocktail. Feces are generally treated in the same manner as the other tissues. Subsequent determination of the percentage of dose excreted in the urine and feces gives an idea of the route, extent and rate of excretion of the drug and the metabolites. The biological samples are then used to profile the parent drug and the metabolites. Estimation of the percentage of each metabolite excreted in the biological samples helps to determine the route of excretion of the metabolites.

Table 4. Percentage of Dose Excreted in Urine, Feces and Bile Following Intravenous Administration of [^{3}H]Indinavir [51]

Species	% of Dose Excreted		
	Urine	Feces	Total
Rat	6	88	94
Dog	3	88	91
Monkey	13	76	89

Several studies describing the procedure for determining the excretion of drugs in animals and humans using [^{14}C] and [^{3}H]labelled compounds have been published [46-50]. Lin and co-workers have described an ADME study of indinavir in rat, dog, and monkey [51]. [^{3}H]Indinavir was dosed intravenously (10 mg/kg) to all three species and the urine and feces was collected over a period of 96 hr. The results indicated that the majority of the dose (>76%) was recovered in the feces and a small fraction (<13%) was found in the urine (Table 4).

Biliary excretion is studied primarily in animals, generally rats and dogs, by surgically implanting a cannula in the common bile-duct of the animals for the collection of the bile and determining the percentage of radioactivity in the bile after intravenous administration. In the above example [51], the biliary excretion of indinavir was determined by dosing bile-duct cannulated rats with 10 mg/kg [^{3}H]indinavir intravenously and bile was collected for 24 hr post dose. About 64% of the dose was recovered in this time suggesting that bile was the major route of excretion (Table 4). If properly conducted to maintain physiological conditions, recovery of bile and urine following an oral dose of radiolabelled drug to bile-duct cannulated animals can yield definative information concerning the extent of absorption.

In our laboratory, we have assessed the effect of bile-duct cannulation on the routes excretion of trovafloxacin in Beagle dogs [52]. After oral administration of 10 mg/kg of [^{14}C]trovafloxacin (specific activity 1.4 to 1.6 µCi/mg) to bile-duct cannulated and non-cannulated dogs the bile, urine and feces (bile-duct cannulated dogs) and urine and feces (non-cannulated dogs) was collected over a period of 72 h. The percentage of dose excreted in the excreta of bile-duct cannulated dogs and non-cannulated dogs is shown in Fig. 5. In bile-duct cannulated dogs, 59, 8.7 and 23.1% of the radioactivity was recovered in the bile, urine and feces, respectively. In contrast, only 2.7% of the radioactivity was recovered in the urine of the non-

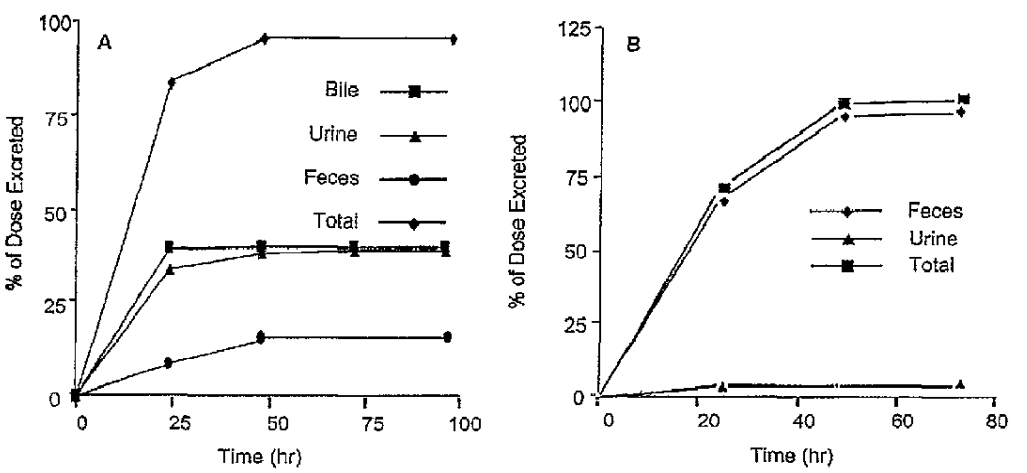


Fig. (5). Excretion of total radioactivity in bile-duct cannulated dogs (A) and non-cannulated dogs after po dose of [^{14}C] trovafloxacin [52].

cannulated dogs. The results of the study clearly indicated that surgical implantation of a bile-duct cannula had altered the excretion pathways of the drug. Thus, radiolabelled trovafloxacin proved to be a valuable tool in demonstrating the effect of surgery on excretion pathways of trovafloxacin.

Incorporation of a radioactive isotope is invaluable in instances where carbon dioxide is expelled as a metabolic product and analyses of the urine and feces fail to yield complete recovery. These experiments are carried out in special metabolism cages in which air is drawn by vacuum pump. Exhaled breath exiting the metabolism cage is passed through an appropriate trapping solution, such as 2-ethoxyethanol and 2-aminoethanol (2:1) which captures $^{14}CO_2$. The trapping solution is replaced and assayed at designated times post-dose that the total the radioactivity expired as labelled carbon dioxide can be determined [53, 54].

Metabolism

A metabolic profile of a drug can provide information of elimination of a compound from the body, facilitate interspecies comparisons and help in designing new drugs. Investigation of metabolism of a compound *in vivo*, requires detection, separation, quantification and isolation of the metabolites produced, compared to metabolism studies using the unlabelled material. The information obtained using a radiolabelled compound is much more definitive and each metabolite is estimated as a percentage of the dose. Historically, biological samples obtained (urine, feces, bile and plasma) following an oral or an iv administration of radiolabelled drug were injected onto a HPLC column either directly or following extraction, to resolve the metabolites and the fractions at various intervals of time were collected. A radioactive profile was then obtained by plotting the counts (in DPM) versus the time (in min). The fractions with high counts were then analyzed by mass spectrometry for metabolite identification. Although this technique is still preferred in cases where low radioactivity levels are present in samples, a more convenient profiling of the radioactive matrices is now obtained by an online radioactivity monitoring detector. Quantitative estimates of the resolved metabolites are thus obtained directly without fraction collection [55]. Metabolites are then isolated and their structures elucidated by HPLC-mass spectrometry (LC/MS), HPLC-tandem mass spectrometry (LC/MS/MS) and multi-nuclear NMR spectrometry and HPLC coupled with high field proton NMR [56].

Fouda and co-workers were the first to report a technique of simultaneously monitoring radioactivity online and acquiring data on a triple quadrupole mass spectrometer, in the same time domain, by a single data system [57]. A schematic diagram describing simultaneous monitoring of radioactivity and mass spectrometric data is shown in Fig. 6. In this technique the post-column effluent is split and introduced into the atmospheric ionization source via the ion-spray interface at 50 µl/min. The remaining effluent is directed into a 500 µl flow cell in the ß-radioactivity monitor. The response of the radioactivity monitor is recorded in real time by the mass spectrometer data system. This provides simultaneous detection of radioactivity and total ion chromatogram. Using this method it is possible to correlate the radioactivity peak with the molecular ions that are observed in the total ion scan, thus simplifying the metabolite identification process. The utility of this

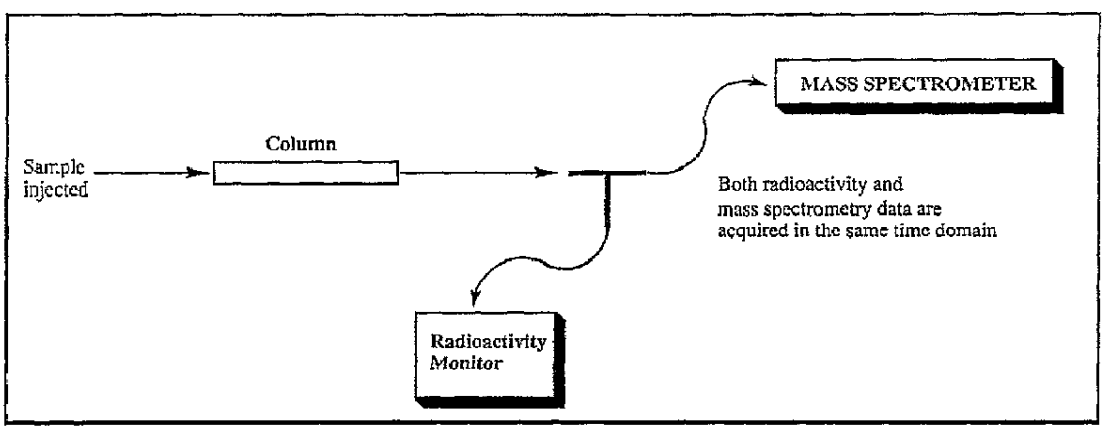


Fig. (6). Simultaneous monitoring of radioactivity and mass spectrometry data [57].

system has been demonstrated by the application in studying the metabolic pathways of several candidates in our laboratory.

As an example, the metabolic fate of droloxifene, a non-steroidal anti-estrogen, in mouse, rat and monkey was investigated using [^{14}C]droloxifene, as shown in Fig. 7 [58]. Droloxifene was extensively metabolized in all three species, primarily by glucuronidation of unchanged drug and oxidative metabolism. Interestingly, the bile of the mouse showed a peak in the radiochromatogram accounting for ~26% of the biliary radioactivity at a retention time of ~5 min. Using the technique described above, we found the signal in the total ion chromatogram corresponding to this radioactive peak gave a molecular ion at *m/z* 756 which was 368 amu higher than the molecular ion of droloxifene. Analysis of the metabolite by MS/MS and NMR suggested that the metabolite was a novel diglucuronide conjugate of an oxidative product of droloxifene.

More recently a new technique, overpressured-layer chromatography combined with digital autoradiography (OPLC-DAR) has been used in the analysis of various biological samples containing radioactivity [59, 60]. This relatively simple and fast purification and isolation tool gives 'practically clean' isolated metabolites suitable for structural identification by different spectroscopic techniques. OPLC is an instrumental version of thin layer chromatography and is suitable for sample application, separation and detection of metabolites

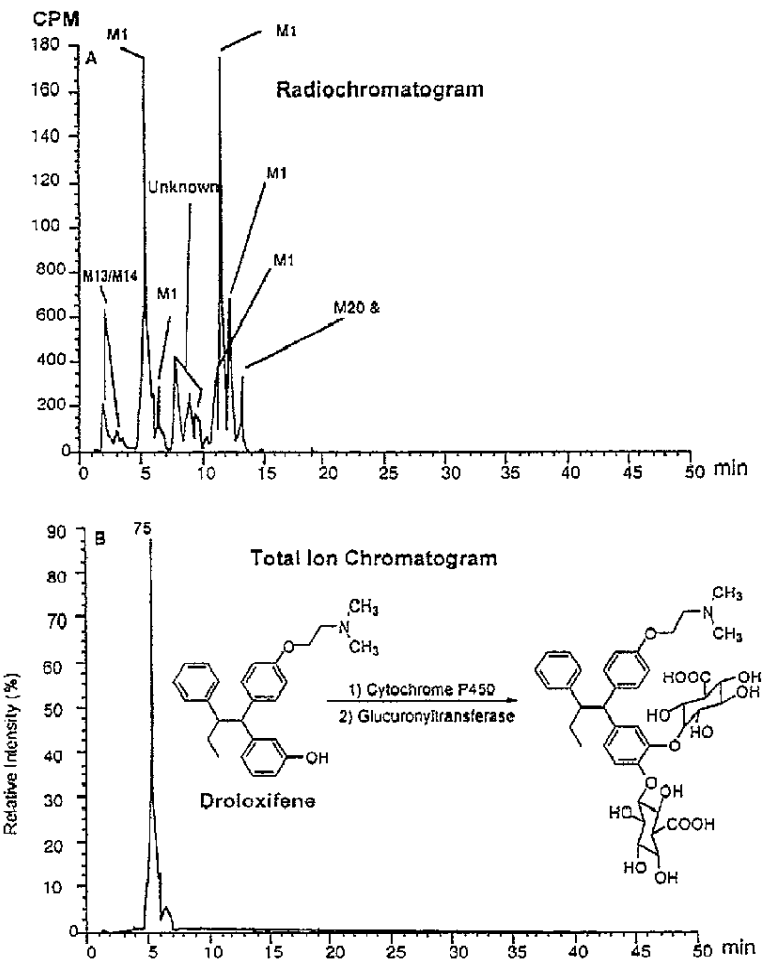


Fig. (7). Identification of droloxifene diglucuronide by simultaneous detection of a peak on the radioactivity monitoring detector and mass spectrometer [58].

both online, by connecting the flow detector or off-line. In the case of OPLC-DAR, the OPLC is combined with digital autoradiography. The technique has been used for quantification and isolation of [^{3}H] and [^{14}C]-deramciclane metabolites. Beagle dogs were treated with 10 mg/kg oral doses of [^{3}H] or [^{14}C]-deramciclane in two separate experiments. Plasma and urine samples were collected and extracted (solid phase extraction). The extracted sample solutions were applied to OPLC plates for isolation or quantification. The structures of the isolated metabolites were then elucidated by mass spectrometry.

Most often, the initial pharmacokinetic studies of a drug in humans are not carried out using radiolabelled material. Although the metabolites in the plasma and urine samples obtained from these subjects can often be identified by means of LC/MS/MS, quantification of these metabolites is difficult unless synthetic metabolites are available as standards. An assessment of the quantity of each metabolite in human matrices (dosed with unlabelled drug) is sometimes possible by comparison of the metabolic profile in human matrices with that in the biological samples of preclinical species dosed with a radiolabelled drug. This was demonstrated in a report on mofegiline metabolism, a monoamine oxidase inhibitor, in dogs and humans [61]. In this study, the metabolites in human urine (dosed with unlabelled drug) were approximately estimated by comparison of the metabolic profiles of urinary extracts from humans and dogs (dosed with a radiolabelled drug). The amount of each metabolite in the dog urine was first estimated by HPLC and an online radioactivity monitoring detector and correlated with the intensity of its molecular ion in the total ion chromatogram, obtained from mass spectrometric analysis. An approximate amount of each metabolite in human urine was then estimated by comparing the retention times and the ion intensities of these metabolites in the total ion chromatograms of dog and human matrices.

Most often the radiolabel is located in a metabolically stable position thereby remaining associated with the metabolites of the molecule. However, failure to place the label in a stable position does not only affect mass balance studies, but can also cause a difficulty in differentiating endogenous compounds from true metabolites of the compound under study. If a drug is known to fragment into two major portions, it is advantageous to trace both fragments by providing each with a different label. The use of the two labels greatly facilitated the tracing and identification of metabolites formed through cleavage of ziprasidone as shown by Prakash and co-workers [62, 63]. An oral dose of a mixture of [^{3}H]- (labelled at the C7 position of the benzothiazole ring) and [^{14}C]- (labelled at C2 position of the ethyl group) ziprasidone was administered to rats, dogs and humans, Fig. 8. The specific activity of ^{14}C-label was 21.8 μCi/mg and ^{3}H-label was 50.0 μCi/mg. Using a mixture of two labels the fragmented metabolites of ziprasidone, [^{14}C]oxindolylacetic acid and [^{3}H]piperazinyl-benzisothiazole could be identified.

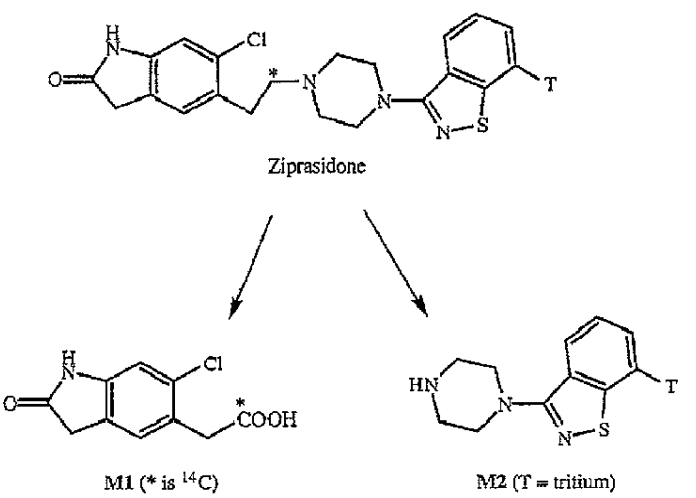


Fig. (8). Identification of metabolites of ziprasidone following administration of a mixture of [^{14}C] and [^{3}H]ziprasidone to rats, dogs and humans [62, 63].

USE OF RADIOISOTOPES IN *IN VITRO* STUDIES

Absorption

In the past decade radioactive tracers have been extensively used to study drug absorption and understand the mechanistic aspects of drug transport across membranes using cell lines such as Caco-2 [64]. A Caco-2 cell line is derived as a monolayer from human colorectal carcinoma. These cells possess many biochemical and morphological properties of an intestinal enterocyte. Radioactive tracers prove to be valuable, especially in instances where a ligand does not possess any UV or fluorescent properties. Typically, the radioligand is introduced on one side of the monolayer and its permeability is assessed by quantification of radioactivity on the other side of the membrane. For example the carrier mediated transport properties of three orally active inhibitors of angiotensin converting enzyme, captopril, enalapril maleate and linospril have been studied using [^{14}C] glycylsarcosine (Gly-Sar) and [^{3}H] Proline as markers by Thwaites and co-workers [65]. The effect of three drugs on the transport of [^{14}C] Gly-Sar, a hydrolysis resistant dipeptide, which is a good substrate for the transporter and [^{3}H]proline was investigated in this study.

It has been demonstrated that the intestinal transporters such as, P-glycoprotein (P-gp) are involved in exsorption of drugs from the blood into the intestinal lumen, thereby influencing drug absorption and bioavailability [66]. Caco-2 cells are reported to express the P-gp efflux pump on the apical side of the cell monolayers after reaching the differentiation stage. Radiolabelled substrates and inhibitors of P-gp such as, [^{3}H]vinblastine, [^{3}H]cyclosporine A and [^{3}H]verapamil of P-gp have been used to determine the affinity of new drugs for P-gp and study the interactions of different compounds with P-gp. Alsenz and co-workers have studied the mechanisms of absorption of the HIV inhibitors saquinavir and ritonavir using [^{3}H]cyclosporin A and [^{3}H]verapamil [67]. The competitive inhibitors increased the net absorption of saquinavir and rotinavir, by decreasing the efflux and increasing influx. More recently, an assay to characterize the competitive interaction and the affinity of drugs and modulators of P-glycoprotein (P-gp) has been developed using [^{3}H]-verapamil as a radioligand [68].

Metabolism

Many metabolism studies are conducted *in vitro* following incubation of the drug with isolated tissues, cells or subcellular fractions (microsomes or cytosol). The use of radiolabelled compounds in such incubations helps to obtain rapid information on the comparative metabolism in different species and to predict the likely routes of metabolism *in vivo*. In some cases, where radiolabelled compounds are difficult to obtain, the incubations are performed using radioactive cofactors. For example, the species differences and possible *N*-glucuronidation of nonpeptide angiotensin II receptor antagonist, SR 47436, was investigated in hepatic microsomes prepared from various species, i.e. Sprague-Dawley rat, Cynomolgus monkey and Caucasian humans, by incubation of unlabelled drug and UDP- [U-^{14}C]glucuronic acid [69]. The role of [^{35}S] has been demonstrated in studying sulfation of various substrates in rat liver cells and human liver cytosol, using either [^{35}S] sulfate or phosphoadenosine-5'-phosphosulfate [PAP^{35}S] as a sulfate donor [70, 71].

Radiotracers of drugs are also valuable in identifying the primary enzymes responsible for the elimination of the drug. Wienkers and co-workers identified the metabolites of tirilazad in rats following incubation of [^{14}C]tirilazad mesylate with rat liver microsomes [72]. Metabolite identification *in vitro* and incubation with specific inhibitors of different cytochrome P450's and cytochrome P450 reductase indicated that CYP2C11 and NADPH dependent 5α-reductase was responsible for metabolizing the drug. A gender difference in tirilazad hepatic clearance was also predicted based on the quantity of the metabolite that was formed in the male and female rats.

DETERMINATION OF ACTIVITES OF DRUG METABOLIZING ENZYMES

The activities of several drug metabolizing enzymes have been determined using specific radiolabelled substrates. A new unified assay for the determination of UDP-glucuronosyltransferase (UGT) activities has been developed using [^{14}C] uridine glucuronic acid and utilizes radioactivity as an end point [73]. One important application of this method is its use in the determination of kinetic parameters for cloned UGT isoforms with greater speed and precision than can be afforded by TLC methodology. Recently, rapid, sensitive and reproducible radioassays using [*N*-methy-^{14}C]-erythromycin, [*O*-methyl-^{14}C]naproxen, [*O*-methyl-^{14}C]dextromethorphan and [3-^{14}C-methyl]caffeine have been developed to determine the activities of cytochrome P450's 3A4, 2C9/10, 2D6 and 1A2, respectively [74-77]. All assays describe the

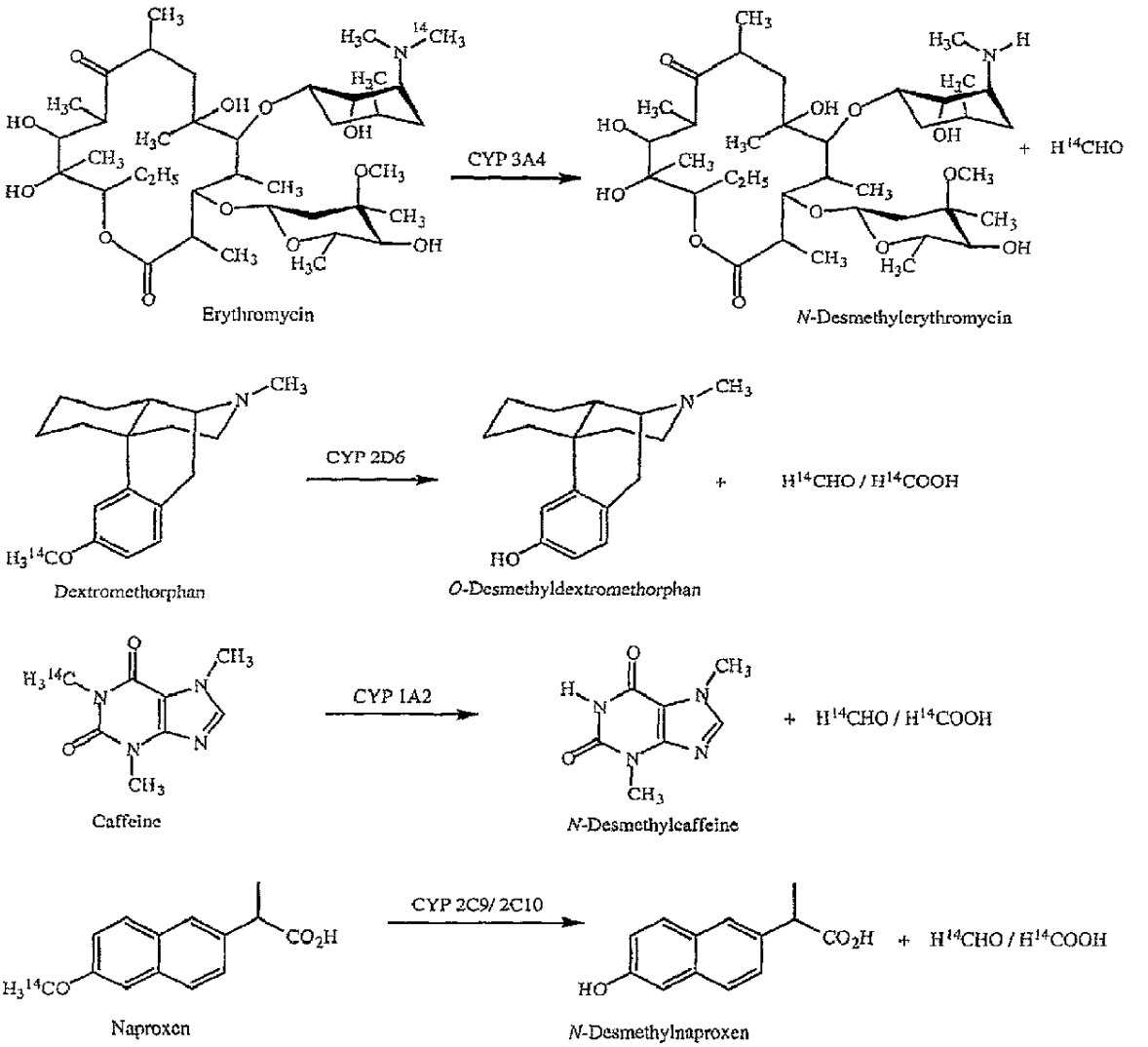


Fig. (9). Oxidative metabolism of [^{14}C-*N*-methyl]erythromycin, [^{14}C-*O*-methyl]dextromethorphan, [^{14}C-methyl]caffeine and [*O*-^{14}C-methyl]naproxen by cytochrome P450 [74-77].

quantification of liberated [^{14}C] formaldehyde/[^{14}C]formic acid following *N*- and *O*-demethylation of substrates, after a single step extraction procedure, using either solid phase or liquid-liquid extraction, Fig. **9**. The ease of quantification of [^{14}C] formaldehyde or [^{14}C]formic acid make these methods suitable for studying drug-drug interactions *in vitro*. A new radioligand binding assay employing [^{3}H]mepyramine has also been reported as a tool to determine the CYP2D1 activity in the rats (can be also used for 2D6 activity in humans) [78]. This compound has a high affinity for the enzyme and drugs that are known to interact with this enzyme either as a substrate or inhibitor, displaced [^{3}H]-mepyramine.

Several studies have been performed with a substrate labelled at metabolically labile positions and the exhaled breath monitored for $^{14}CO_2$ content in order to examine the metabolic disorder. More recently, a $^{14}CO_2$ breath test has been used to determine the CYP 3A4 and 2E1 activities using

[*N*-methyl-^{14}C] erythromycin and [^{14}C] nitrosodimethylamine in humans and rats, respectively [79, 80]. In both cases, these non-invasive assays measure exhaled $^{14}CO_2$ resulting from *N*-demethylation of the radiolabelled substrates.

USE OF RADIOISOTOPES IN TOXICOLOGY

Teratology Studies

Placental transfer studies with [^{14}C]labelled compounds provide information concerning potential tetratogenicity. The amount of compound reaching the fetus, the rate of transfer to fetal circulation, and the half-life in the fetus are some parameters required by the regulatory authorities during filing of the NDA for the approval of a new drug substance. Tetratology studies have been historically carried out by harvesting the fetuses on gestation days following administration of a radiolabelled compound and determining the radioactivity in these fetuses using the procedure described for TD/LSC. In the past decade however, these studies are commonly carried out using WBA techniques [81, 82]. Herman and Chay have illustrated this by estimating the fetal exposure of LY217896 in the placenta and fetuses of rabbits following administration of [^{14}C]LY217896 on gestation day 18 [83]. WBA method has also been used to demonstrate the species difference in the distribution of a teratogenic thalidomide analog EM12 in the early embryo of marmoset monkey, Wistar rat and NMRI mouse [84]. Administration of thalidomide and its analogs during early gestation is known to result in specific and dramatic limb defects in primates. However, such defects are not observed in rats and mice. The authors used radiolabelled EM12 since it was metabolically more stable and much more teratogenic in the monkey. The distribution in the embryo; including the target tissue, the embryonic limb bud was examined in three species by WBA. The results indicated that the exposure to radioactivity was greater in the monkey embryo than in the rat and mouse embryo and that the accumulation of radioactivity in limb buds and neural epithelium of monkey embryo was much more pronounced relative to other areas.

Mechanisms of Toxicity

In recent years, the radioisotopes have been increasingly used to understand mechanisms of toxicity produced by xenobiotics. Metabolism of exogenous compounds is generally thought to serve as a detoxification pathway by facilitating the excretion of compounds as polar metabolites. However, it has become clear that compounds are not often toxic themselves but require metabolic transformation to a reactive intermediate to exert toxicity. Irreversible binding of a reactive intermediate to macromolecules (proteins and nucleic acids) in the body have been said to induce biochemical and physiological changes resulting in cell damage, cell death, mutagenesis or tumorigenesis.

Bioactivation of xenobiotics to potentially hepatoxic metabolites is frequently investigated by incubating the drug with hepatic subcellular fractions or an enzyme system. The presence of a radioactive isotope in the drug candidate proves to be a very valuable tool for conducting these studies. If the compound is radiolabelled, the formation of the reactive metabolite can be assessed by estimating the amount of radioactivity that is covalently linked to proteins in the incubation mixture. For example, the conversion of L-746,530 and L-739,010 to reactive metabolites and the mechanism of activation of these compounds to their toxic intermediates using [^{14}C]labelled tracers of the two compounds has been investigated by Chauret and co-workers [85, 86]. Following incubation of the radiolabelled compound with hepatic microsomes and NADPH, the proteins were precipitated and washed. The radioactivity associated with microsomal proteins was quantified by dissolving in sodium hydroxide and subsequent scintillation counting. In another example, incubation of [^{14}C] delavirdine with microsomes from several species resulted in irreversible association with microsomal proteins [87]. Irreversible binding of [^{14}C] delavirdine to microsomal proteins was determined by incubating the radiolabelled drug with the microsomes followed by centrifugation of the protein pellet. The pellet was then dissolved in 500 µl of 0.1% SDS, vortexed vigorously and reprecipitated by addition of ice-cold methanol. These washing steps were repeated for an additional two times in order to wash out entrapped or unbound radioactivity. The radioactivity in the pellets was then counted by dissolving in SDS and mixing with the scintillation cocktail.

In many cases, incubation with a radiolabelled compound can be useful in identifying the protein(s) that are involved in adduct formation as well as the sites of covalent binding. In both examples described above, irreversible binding of L-746,530 and delavirdine to an approximately 50 kDa protein was shown using SDS-

PAGE/autoradiography [85, 87]. Kent and co-workers investigated the inactivation kinetics of purified reconstituted CYP 2B1 by N-[^{14}C]-7-benzyl-1-aminobenzotriazole (BBT) [88]. The inactivated protein was resolved by SDS-PAGE/autoradiography and the association of radiolabel with the apoprotein was shown. In an other example, mechanism based inactivation of 2B1 by 9-ethynylphenanthrene (9-Eph) was investigated using 2-[^{3}H]-9Eph [89]. A radiolabelled peptide of approximately 3.0 kDa was identified by autoradiography after Tricine SDS-PAGE analysis of the peptide fragments, generated from cyanogen bromide cleavage of [^{3}H]-EPh-inactivated P 450 2B1. HPLC fractionation and analysis of the fraction containing the most radioactivity by matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) suggested the site to be one of the amino acids contained in the peptide Phe 297 to Leu 307.

The high sensitivity that is achieved by using radiolabelled tracers is useful in determining the terminal phase of low but long lasting blood concentrations of drug-related material following an intravenous or oral dose of drugs to animal species or humans. The estimation of blood concentrations at very low levels results in long half-lives and often represent elimination of the slowest radiolabelled metabolite that is reversibly bound to and slowly released from a specific tissue depot. Comparison of the half-lives of radiolabelled drug-related material and the unchanged parent drug can sometimes help in assessing possible covalent interaction of drug-related material with plasma proteins. The amount of radioactivity bound to plasma proteins is estimated in the same way as done for microsomal proteins. The bioactivation of L-739,010 and the covalent binding of reactive metabolites to plasma proteins was investigated by Zhang and co-workers *in vivo* following intravenous administration of [^{14}C]-L-739,010 to rhesus monkey and rat [90]. The half-lives of the radiolabelled material were 2.7 and 4 days, respectively, in these two species, while those for the parent drug were only about 3 h. The plasma radioactivity at later time points could not be extracted by organic solvents suggesting that L-739,010 produced reactive metabolite(s) that bound covalently to plasma proteins.

Hepatic protein targets of reactive metabolites of acetaminophen in mice (*in vivo*) have been recently identified using radiolabelled acetaminophen, gel electrophoresis and a new mass spectrometry based strategy [91]. Radiolabelled acetaminophen was administered to mice and the proteins in the liver tissue lysate were separated by 2-dimensional PAGE. In-gel digestion of the radiolabelled gel spots gave a set of tryptic peptides that were analyzed by MALDI-MS. The identities of the modified liver proteins were then determined by comparison of the experimentally derived molecular weights with those in the data bases resulting in the identification of twenty new drug labelled proteins.

^{32}P-Postlabelling

A majority of chemical carcinogens bind covalently to DNA and RNA usually after bioactivation causing alteration in the genetic material ultimately leading to cancer. Generally these carcinogen-DNA adducts are formed in very low levels and require ultra-sensitive methods for their detection and quantification. Radioactive carcinogens have been quite commonly used in characterizing the DNA adducts and have provided important information about the interactions between carcinogens and DNA [92]. In the early 1980's, Gupta and co-workers used ^{32}P in the analysis and detection of the DNA adducts [93, 94]. In this approach, the ^{32}P was incorporated into DNA constituents after exposure of the DNA to a non-radioactive binding chemical [95]. The evidence for the presence of chemically altered nucleotides was then provided by the appearance of extra spots of the digests of chemically modified DNA as detected by autoradiography of TLC's. Since the development of this assay, it has been widely used to detect and quantify DNA adducts of several carcinogens and procarcinogens that undergo bioactivation.

In the past few years, this technique has been used in identifying tamoxifen induced DNA adducts. Tamoxifen is widely used in the treatment of breast cancer and is known to induce hepatocellular carcinomas and adenomas in rats. Although the compound appears to be inactive in the conventional genotoxic screen, it readily forms DNA adducts in rat, mouse and hamster liver upon bioactivation by cytochrome P450. A recent report by Koskinen and co-workers demonstrated the use of ^{32}P-postlabelling in identifying DNA adducts that where induced by tamoxifen [96]. DNA isolated from livers of rats receiving tamoxifen was subjected to ^{32}P-postlabelling and the postlabelled DNA hydrolysis mixture was analyzed by reversed-phase HPLC with ^{32}P online detection and by TLC on polyethyleneimine plates, followed by autoradiography. Recently, a new *in vitro* model combining the short term incubation of precision-cut liver slices with DNA-adduct analysis by the ^{32}P-postlabelling technique for investigating

genotoxic potential of xenobiotics was reported [97].

^{32}P-Postlabelling technique has also found application in studying the metabolism of macromolecules. For example, the metabolism of an [^{35}S] labelled antisense oligodeoxynucleotide phosphothiorate, GM91® was investigated in cynomolgus monkey, by polyacrylamide gel electrophoresis after extracting the biological samples and subjecting them to [^{32}P] postlabelling (either 3'-end or 5'end) [17].

CONCLUSION

The present report summarizes the role and the impact of radioisotopes in conducting ADME and toxicology studies. Although the concepts of quantification and detection of radioactivity have not changed, new technologies and strategies in designing ADME studies using radiotracers have emerged. It must be realized that this report of new developments and applications of radioactive tracers is by no means complete but rather a synopsis of the use of radiolabelled compounds in the area of drug metabolism and disposition.

Recently, new enabling technologies, such as accelarator mass spectrometry (AMS) [98], and liquid chromatography-chemical reaction interface mass spectrometry (LC/CRIMS) [99-101] have provided an alternative approaches to study the disposition of new drug candidates. Also, increasing restrictions on the disposal of radioactive waste has added additional pressure to curtail the use of radiolabelled compounds. However, in spite of these alternative approaches and the restrictions, the use of radiotracers in pharmacological research provides vital information which cannot practically be obtained by other means. Indeed, the enhanced need for detailed ADME data and assessment of potential reactive intermediates at earlier stages of drug candidate development has served to further promote their utilization.

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